REMARKS

The present application is directed to a method for detecting one or more biological entities in a sample and for obtaining information resident in the genetic code of a biological entity in a sample. The method is particularly useful for the detection or characterization of a pathogen, such as a biological weapon. Currently, no broad spectrum, high confidence, identification or characterization systems that yield low false positive detection rates are available. The method described in the present application is useful for simultaneously determining the presence or absence of thousands of informative nucleic acid sequences from an entire sample, thereby providing rapid, meaningful results. The present method is specific, yet has minimal false positive results, thereby providing a high degree of confidence in the results obtained because of the high magnitude of genetic sequence and related information than can be provided simultaneously. Furthermore, the present method can be used to identify signs of genetic engineering in an organism, to characterize unknown or previously unidentified organisms, and to provide information concerning virulence, antibiotic resistance or other DNA features of the biological entity. In summary, the present invention provides novel methods for simultaneously detecting the presence of hundreds of known and unknown pathogens potentially present in a sample. Clearly, such a method is seriously needed.

The present application is a continuation application of U.S. Patent Application Serial No. 09/563,038 filed May 1, 2000. Claims 1-15 have been canceled and Claims 16-140 are pending. Favorable consideration of the currently pending claims is respectfully requested in light of the following remarks.

Rejection of Claims under 35 U.S.C. § 103(a) as obvious over Telenius in view of Peng

In the Office Action mailed January 31, 2003 in U.S. Patent Application Serial No. 09/563,038, the parent of the present application, the Examiner rejected Claims 86-90, 92, 93, 96, 97, 99, 101-106, 108, 109, 112, 113, 115, 117-122, 124, 125, 128, 129, 131, 133-138, 140, 141, 144, 145, 147, 149-154, 156, 157, 160, 161, 163 and 165 under 35 U.S.C. § 103(a), as unpatentable over the scientific paper of Telenius *et al.*, *Genomics* (1992) 13:718-725

(hereinafter "Telenius") in view of the scientific paper of Peng, *et al.*, *J. Clin. Pathol.* (1994) 47:605-608 (hereinafter "Peng") on the basis that it would be obvious to replace the degenerate primers used in the DNA amplification method taught by Telenius with the random primers taught by Peng to achieve the method claimed in the present application. Applicants respectfully traverse.

The present application claims a method for detecting a biological entity in a sample by combining the sample with random primers, amplifying the sample nucleic acid sequences using a polymerase chain reaction (PCR), hybridizing the amplification products to an array of predetermined nucleic acids and detecting the hybridized amplification products. The claimed method allows for the rapid analysis of a test sample with a high degree of accuracy.

The references cited by the Examiner fail to teach a biological entity **detection or identification** method as claimed in the present application. The cited references merely teach various methods for the **amplification** of nucleic acid molecules.

Telenius teach a nucleic acid amplification method using **degenerate** oligonucleotide sequences as primers to amplify sample DNA. The amplified DNA is labeled by reamplification in the presence of biotinylated dUTP, and the extent of amplification is determined by hybridization of the amplification products to normal male metaphase chromosomes on microscope slides. (See Telenius at page 719, column 1, lines 27-45.) The degenerate primers contain a restriction enzyme cleavage site at the 5' end and at least three specified bases at the 3' end to encourage the primers to bind to and amplify DNA sequences commonly found in nature.

Peng teach a nucleic acid amplification method in which **random** primers are used to amplify sample DNA. The Peng amplification method simply generates more starting material for subsequent analysis by standard PCR. (See Peng, page 605, left column, lines 13-16, below the abstract.) Therefore, Peng is simply an amplification system for use in circumstances when sample size is extremely limited. To achieve amplification without introducing numerous copy errors, Peng uses a multistep system. In Phase I of the amplification, a reduced amount of polymerase is combined with the sample and modified amplification cycles performed. In Phase II of the amplification, an additional amount of polymerase and reagents are added to the

amplification product of Phase I and standard amplification cycles are employed. No detectable molecule is incorporated into the amplified product and no hybridization techniques are employed. (See Peng at page 605, right column, line 38 to page 606, left column, line 8.) The resulting amplified DNA is then subjected to conventional PCR using **specific matched primer pairs**. (See Peng at page 606, left column, lines 17- 38, in the paragraph entitled "Multiple PCR Analyses." The PCR results, obtained using the specific **matched primer pairs**, are then analyzed using agarose gel electrophoresis. Accordingly, the Peng amplification process results in highly preferential sites which are dictated by the sequences of the matched primer pairs where start and stop lengths are specified. Furthermore, as demonstrated below, the Peng process results in the amplification of non-random lengths. Therefore, this results in Peng failing to amplify the entire sequence of the genome.

Applicants respectfully submit that the cited references fail to disclose all the elements of the claimed method, either alone or in combination, as described in more detail below. Even if all the elements of the claimed method were disclosed by the cited references, which Applicants do not concede, one skilled in the art would lack the motivation to combine the amplification methods of the cited references to produce the claimed detection method. In fact, the cited references teach away from each other. Furthermore, if in some way the cited references could be combined to arrive at the claimed method, which Applicants maintain is impossible, the claimed method achieves unexpected results such as the simultaneous detection of thousands of nucleic acid sequences with a high degree of confidence thereby providing rapid information that can be used to identify a pathogen or multiple pathogens, to identify evidence of genetic engineering in an organism, to characterize unknown or previously unidentified organisms, and to provide information concerning virulence, antibiotic resistance or other DNA features of the biological entity. In addition, the claimed method resolves a long felt need by the scientific community and the military for a biological entity detection method useful for the detection of deadly pathogens such as bioterrorism or biowarfare agents.

Cited references fail to disclose all elements of claimed method

Applicants respectfully submit that the cited references, either alone or in combination, fail to disclose all the elements of the claimed method. All of the claims in the present application require 1) combining nucleic acid sequences in a sample with multiple primers of randomized nucleotide sequences, 2) amplifying sample nucleic acid sequences to produce amplification products, and 3) hybridizing the amplification products to an array of predetermined nucleic acid sequences.

Both applicants and the Examiner agree that Telenius teaches the use of **specific** primers and, therefore, fails to teach the use of **random** primers for nucleic acid amplification. Claims 16, 32, 48, 80, 96, and 125 of the present application specify that nucleic acid sequences in a sample are combined with multiple primers of **randomized** nucleotide sequences. Telenius also fails to teach the hybridization of amplification products to an array. Instead, Telenius hybridizes the amplified products to normal male metaphase chromosomes immobilized on a microscope slide and uses a fluorescence *in situ* hybridization (FISH) technique to determine the extent of amplification for genome mapping purposes. Claims 16, 32, 48, 80, 96, 125 of the present application specify that the amplification products are hybridized to an array and Claims 28, 44, 60, 76, and 92 specify that the predetermined nucleic acid sequences are at predetermined positions on the array wherein the nucleic acid sequences at two or more predetermined positions characterize a different biological entity or variant of a biological entity.

Peng fails to teach the use of random primers as claimed. Claims 16, 80, 96 and 125 of the present application specify that the random primers employed in the claimed method are "sufficiently randomized to provide nonpreferential start sites for amplification of the sample nucleic acid sequences." As clearly admitted by Peng, their "random" primers **fail** to amplify the fragment of p53 exon 7-9 (840 base pairs) from any of the amplified pools (See Peng, page 606, right column, lines 49-51.) Therefore, unlike the claimed method, the "random" primers used by Peng have **preferential** starts sites. In addition, Claims 48, 64, 80 and 126 specify that "substantially an entirety of the nucleic acid sequences of the biological entity are represented among amplification products." Clearly Peng fails to amplify substantially all sample nucleic

acid sequences as evidenced by the statement on page 606, right column, lines 49-51, that the 840 base pair fragment could **not** be amplified.

In addition, Peng fails to teach incorporation of a detectable nucleoside triphosphate during **random primer** amplification to produce detectable amplification products as specified in Claims 136-140. Peng is unable to introduce a label into the amplification product until the second **specific** PCR amplification, which is at least the fourth step of the method. (See Peng, page 606, lines 48-54.)

Furthermore, Peng fails to teach hybridization of random primer amplification products to an array. As mentioned above, the random primer amplification products produced by Peng are not detectable and are not analyzed directly. The random primer amplification products are subjected to specific primer PCR, and these specific primer amplification products are subjected to a second specific primer amplification step to introduce a label. The labeled, specific primer PCR amplification product is **then** run on an acrylamide gel and exposed to x-ray film to produce the films shown in Figure 2 of Peng.

Cited references lack motivation to combine

In addition to the assertion that neither Peng nor Telenius teach all the elements of the claimed method, Applicants respectfully submit that one skilled in the art would lack the motivation to combine the amplification methods of Telenius and Peng to produce the claimed detection method. Both Telenius and Peng were developed for the amplification, not the detection, of DNA. The method of Telenius was developed for the amplification of **pure** DNA. (See Telenius at page 718, column 2, line 39 to page 719, column 1, line 7.) The method of Peng was developed for the amplification of **trace amounts** of DNA. (See Peng at page 605, right column, lines 12-15.) In contrast, the claimed method was developed for the rapid detection and characterization of one or more target nucleic acids from one or a **multitude** of pathogenic organisms, such as in complex biological samples. The claimed method rapidly amplifies, labels **and detects** the target with minimal risk of false positive results. Neither Telenius, nor Peng, alone or in combination, envisioned such a simple, rapid and versatile detection system.

Cited references teach away from each other

Peng not only reject the teachings of Telenius, they actually **teach away** from Telenius as being **inadequate** for the type of amplification they wish to achieve. The authors of the Peng paper cite the Telenius paper as describing a method for the amplification of genomic DNA, but imply that the Telenius method lacks simplicity and reliability for the amplification of **minute** quantities of DNA. (See Peng, page 605, line 7 below the abstract to column 2, line 4 in which Telenius is cited as reference 2.) Therefore, one skilled in the art would lack the motivation to **combine** the teachings of Telenius and Peng because Peng teach that their amplification method is a complete **substitute** for the method of Telenius.

In addition, Peng caution that the use of random primers can create efficiency/fidelity problems. Therefore, they devised an elaborate, two-step amplification process to minimize errors in the DNA copying process. (See Peng at page 607, column 2, lines 9-28.) One skilled in the art seeking to develop a **rapid** DNA detection method would avoid a combination of the **multi-step** amplification method of Peng with the **multi-step** amplification and labeling method of Telenius due to the length of time required to complete all of the combined steps.

Applicants further submit that one skilled in the art would fail to combine the references because Telenius **teach away** from the use of the random primers for DNA amplification. Telenius use a combination of degenerate and specific bases in their probes and state that the use of primers having six specified bases at the 3' end resulted in more efficient amplification than the use of primers having only three specified bases at the 3' end. (See Telenius at page 719, column 2, line 59 to page 720, column 1, line 2). Therefore, according to Telenius, the more **specified** bases, the better. The concept of replacing any or all of the degenerate or specific bases with random bases is in complete opposition to the teachings of Telenius.

Claimed method achieves unexpected success

Even if the Peng and Telenius amplification methods could be combined in some way, which applicants maintain they do not, applicants respectfully submit that the claimed detection method achieves unexpected results.

For example, the method claimed in the present application can determine the presence or absence of thousands of nucleic acid sequences in the sample in a massively parallel fashion. In this way, the biological entity or entities are detected very rapidly. The importance of speed for the identification or characterization of a biological entity having the capacity for widespread biological infection is clearly understood. Claims 28, 44, 60, 76, and 92 describe the array as having nucleic acid sequences at two or more predetermined positions with different biological entities or variants of a biological entity, which allows the method to detect more than one entity per assay as claimed in Claims 29, 45, 61, 77, 93, 98 .

The claimed method is able to identify hundreds or thousands of sequences per biological entity and is therefore highly sensitive, yet exhibits minimal false positive results due to redundancies provided on the array, as claimed in Claim 113. This provides an unexpectedly high level of confidence not enjoyed by currently available detection methods, such as standard PCR assays, which are limited to only a few sequence-based questions per assay.

The claimed method even has the ability to characterize previously unidentified organisms as claimed in Claim 104 and can provide phylogenetic information as claimed in Claim 105. In addition, the claimed method is able to identify signs of genetic engineering in biological organisms and can characterize the biological entity in terms of virulence factors, antibiotic resistance, transmissibility, or other nucleic acid features as claimed in Claims 106-112. By way of example, the present method could be used to detect the presence of a genetically modified organism such as a salmonella pathogen that had been modified to incorporate the shigatoxin gene of E-coli 0157:H7.

For at least the above reasons, applicants respectfully request that the Examiner withdraw the rejection.

Rejection of Claims under 35 U.S.C. § 103(a) as obvious over Peng in view of Telenius and further in view of the Boehringer-Manheim catalog

In the Office Action mailed January 31, 2003 in U.S. Patent Application Serial No. 09/563,038, Claims 91, 94-95, 107, 110-111, 123, 126-127, 139, 142-143, 155, and 158-159 were rejected under 35 U.S.C. §103(a) as being obvious over Peng, in view of Telenius, as

described above, and further in view of the Boehringer-Manheim catalog (1998) pages 70-76. Applicants respectfully traverse this rejection.

The claims of the present application corresponding to the rejected claims depend directly or indirectly from independent Claims 16, 32, 48, and 80 and contain all the limitations therein. The claims of the present application corresponding to the rejected claims further specify the incorporation of a detectable molecule into the amplified nucleic acids or methods of detecting a detectable amplification product.

The Boehringer-Manheim catalog teaches nucleic acid labeling by the incorporation of radioactive, fluorescent, and digoxigenin labels.

As discussed above, one skilled in the art would lack the motivation to combine the teaching of Peng with the teachings of Telenius, nor is there an expectation of success if such teachings could be combined that they would yield applicants' claimed method. Therefore, the arguments presented above with regard to the rejections based on Telenius and Peng are repeated here.

Applicants respectfully submit that the additional teachings of the Boehringer-Manheim catalog fail to rectify the shortcomings of the teachings of Peng and Telenius. The combination of all three references fails to yield a teaching that creates a case of prima facie obviousness of applicants' currently pending invention. Therefore, applicants respectfully request that the Examiner withdraw this rejection.

Rejection of Claims under 35 U.S.C. § 103(a) as obvious over Peng in view of Telenius and further in view of Beattie

In the Office Action mailed January 31, 2003 in U.S. Patent Application Serial No. 09/563,038, Claims 98, 114, 130, 146 and 162 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Peng, in view of Telenius, as described above, and further in view of Beattie *et al.*, PCT application International Publication Number WO 97/22720 (hereinafter "Beattie"), which corresponds to U.S. Patent No. 6,156,502. Applicants respectfully traverse.

Claims 28, 44, 60, 76 and 92 of the present application specify that the predetermined nucleic acid sequences are at predetermined positions on the array and that the nucleic acid

sequences at two or more predetermined positions characterizes a different biological entity or variant of a biological entity.

Beattie teaches a variety of methods involving the amplification of DNA from an organism, tissue or cells, followed by hybridization of the amplified DNA to a two-dimensional array of oligonucleotide probes to form a hybridization fingerprint. The fingerprint is then compared with the hybridization fingerprint of a known organism to provide identification of the organism.

Applicants respectfully submit that neither Telenius, Peng, nor Beattie, alone or in combination, teach, suggest or imply hybridization of amplified nucleic acids to predetermined nucleic acids on an array as claimed in Claims 28, 44, 60, 76 and 92. As mentioned above, Peng fails to disclose hybridization at all, and Telenius amplifies human genomic DNA and analyzes the results using a fluorescence *in situ* hybridization (FISH) technique to determine the **extent** of amplification, not the identity of the nucleic acids in the sample. By using an array that contains nucleic acid molecules from different biological entities or variants of biological entities, applicants are able to quickly identify a biological entity in the sample, such as anthrax in an environmental sample suspected of contamination by a biological weapon, and take immediate steps to contain or treat the contamination to prevent further transmission. Unlike conventional weapons, biological weapons continue to kill or destroy long after their initial deployment. When contamination by a biological agent is identified quickly, the extent of damage from the biological weapon is minimized.

The method described in Claims 28, 44, 60, 76 and 92 provides an array containing different biological entities or variants of a biological entity, so that a single test provides immediate meaningful information. In addition, the detection method is self-verifying and provides the user with a high degree of confidence that a result is not a false positive result.

For at least the foregoing reasons, applicants respectfully request that the Examiner withdraw this rejection.

Rejection of Claims under 35 U.S.C. § 103(a) as obvious over Peng in view of Telenius and further in view of Sayada

In the Office Action mailed January 31, 2003 in U.S. Patent Application Serial No. 09/563,038, Claims 100, 116, 132, 148 and 164 were rejected under 35 U.S.C. § 103(a) as being obvious over Peng, in view of Telenius, as discussed above, and further in view of Sayada *et al.*, *Electrophoresis* (1994) 15:562-565 (hereinafter "Sayada"). Applicants respectfully traverse this rejection.

The rejected claims depend directly or indirectly from independent Claims 16, 32, 48, and 80 and contain all the limitations therein. The claims of the present application corresponding to the rejected claims further specify that the biological entity to be detected in the sample is a pathogen.

Sayada teaches the use of PCR in the analysis of pathogenic organisms.

As discussed above, one skilled in the art would lack the motivation to combine the teachings of Peng with the teachings of Telenius, nor is there an expectation of success if such teachings could be combined that they would yield applicants' claimed method. Therefore, the arguments presented above with regard to the rejections based on Telenius and Peng are repeated here.

Applicants respectfully submit that the additional teachings of Sayada fail to rectify the shortcomings of the teachings of Peng and Telenius. The combination of all three references fails to yield a teaching that creates a case of prima facie obviousness of applicants' currently pending invention. Therefore, applicants respectfully request that the Examiner withdraw this rejection.

Claimed method resolves long felt need for detection method

As described in the enclosed Declaration of R. Paul Schaudies and Doreen A. Robinson Under 37 C.F.R. §1.132 (hereinafter "the enclosed Declaration"), a critical, urgent need for a rapid and reliable method for detecting biological entities has existed for over fifty years. Currently available detection methods simply identify a biological attack by epidemiological means. For example, hospital and other medical or public health workers report abnormally high numbers of unusual infections. Based on the symptoms of the ill or autopsy reports of the dead,

several diagnoses are proposed and conventional assays, such as PCR analysis, are used to confirm or negate the proposed biological entities as the cause of the infections, one by one. These assays are slow and often unreliable. Many individuals are infected, ill or even dead before the outbreak is even discovered.

As mentioned in the enclosed Declaration, the first recently recognized major attack using biological weapons in the United States was by a religious cult in Oregon in 1984. Cult members contaminated salad bars in local restaurants with Salmonella in an attempt to influence the results of a political election. Although the organism was identified as Salmonella due to the symptoms of the victims and subsequent culturing, scientists concluded that outbreak was caused by accidental contamination by restaurant staff. The true cause of the attack was never confirmed scientifically. The individuals responsible for the attacks were convicted by the testimony of other cult members and evidence such as purchase orders of Salmonella cultures. The need for a biological weapon detection method was clearly evident.

By 1991, no biological detection method enabling broad spectrum, high confidence, and low false positive rates were yet available. The proposed solution for a biological weapon detection method was to use available technologies on a massive scale. Seven years after that, as recently as 1998, still no biological weapon detection method as technologically advanced as the presently claimed invention existed. The development of "massive laboratory detection and characterization capabilities" along with "data-sharing between scientists on the Internet" were the proposed solutions.

The United States was and continues to be vulnerable to biological attack as clearly illustrated by the transmission of anthrax-laced letters to several individuals in 2001 through the U.S. Mail. This attack, which resulted in five deaths and several illnesses, demonstrated that massive disruption and damage could result from a biological attack using extremely simple delivery methods and small amounts of biological material. U.S. leaders and citizens realized that the public health risks, government costs and adverse economic consequences of a biological attack were enormous.

The U.S. Government has clearly recognized the need for the development of such a detection method over the last six years by steadily increasing appropriations for programs such as the U.S. Department of Defense Chemical and Biological Defense Program, which reached an unprecedented 1.3 billion dollars in 2003.

Even the references cited by the Examiner echo the long felt need for a biological entity detection method. These references have been available for a long period of time during which enormous advances have been made in genetic techniques. However, no broad spectrum, high confidence, and low false positive rate biological detection method has emerged. Telenius was published over twelve years ago. Peng and Sayada are both over ten years old. Beattie was originally filed over eight years ago. Even the Boehringer-Manheim catalog is at least six years old.

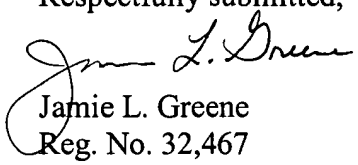
Applicants respectfully submit that scientists have been searching for a method for the detection or characterization of a biological entity in the event of a biological warfare attack without success until the discovery of the method described in the present application.

Conclusion

Applicants respectfully submit that the claims are non-obvious and are in condition for allowance. Early and favorable consideration is earnestly solicited. If the Examiner believes there are other issues that can be resolved by telephone interview, or that there are any informalities remaining in the application that may be corrected by Examiner's Amendment, a telephone call to the undersigned attorney at (404) 745-2473 is respectfully solicited. Should any

additional fees be required in connection with the filing of this response, the Commissioner is hereby authorized to charge the same to Deposit Account No. 501458.

Respectfully submitted,



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